

FIG. I

DNA sequence for human preproparathyroid hormone.

10 30 50
ATGATHCCNGCNAARGAYATGGCNAARGTNATGATHGTNATGYTNGCNATHTGYTTYYTN

70 90 110
ACNAARWSNGAYGGNAARWSNGTNAARAARMGNWSNGTNWSNGARATHCARYTNATGCAY

130 150 170
AAYYTNGGNAARCAYYTNAAYWSNATGGARMGNGTNGARTGGYTNMGNAARAARYTNCA

190 210 230
GAYGTINCAAYATTYGTNGCNYTNGNGNCNCCNYTNGCNCCNMNGAYGCNGGNWSNCAR

250 270 290
MGNCCNMGNAARAARGARGAYAAYGTNYTNGTNGARWSNCAYGARAARWSNYTNGGNGAR

310 330
GCNGAYAARGCNGAYGTNAAYGTYNACNAARGCNAARWSNCARTRR

M	=	A	OΣ	C
R	=	A	OΓ	G
W	=	A	OΣ	T
S	=	C	OΣ	G
Y	=	C	OΣ	T
H	=	A	OΣ	C OΣ T
N	=	A	OΣ	G OΣ C OΣ T.

FIG. 2

DNA sequence for human
preproparathyroid hormone in plasmid pSSHPTH-10.

10 30 50
ATGATGATAACCTGCAAAAGACATGGCTAAAGTTATGATTGTATGTTGGCAATTGTTTT

70 90 110
CTTACAAAATCGGATGGAAATCTGTTAAGAACAGAGATCTGTGAGTGAAATACAGCTTATG

130 150 170
CATAACCTGGGAAACATCTGAACTCGATGGAGAGAGTAGAAATGGCTGCCGTAAAGAACGTC

190 210 230
CAGGATGTGCACAATTGTTGCCCTTGGAGCTCCTCTAGCTCCCAGAGATGCTGGTTCC

250 270 290
CAGAGGCCCGAAAAAGGAAGACAATGTCTTGGTTGAGAGCCATGAAAAAGTCTTGG

310 330
GAGGCAGACAAAGCTGATGTGAATGTATTAACCTAAAGCTAAATCCCAGTGA

FIG. 3

Portion of DNA sequence of the plasmid
for insertion into E. coli, coding for human
preproparathyroid hormone with flanking sequences.

10 30 50
TATGATGATHCCNGCNAARGAYATGGCNAARGTNATGATHGTNATGYTNGCNATHGYTT

70 90 110
YYTNACNAARWSNGAYGGNAARWSNGTNAARAARMGNWSNGTNWSNGARATHCARYTNAT

130 150 170
GCAYAAYYTNGGNAARCAYYTNAAYWSNATGGARMGNGTNGARTGGYTNMGNAARAARYT

190 210 230
NCARGAYGTNCAYAAYTTYGTNGCNYTNGGNGCNYTNGCNCNMNGAYGCNGGNWS

250 270 290
NCARMGNCCNMGNAARAARGARGAYAAYGTNYTNGTNGARWSNCAYGARAARWSNYTNGG

310 330 350
NGARGCNGAYAARGCNGAYGTNAAYGTNYTACNAARGCNAARWSNCATRRAAATGAAA

370 390 410
ACAGATATTGTCAGAGTTCTGCTCTAGACAGTGTAGGGCAACAATAACATGCTGCTAATTCA

430
AAAGCTCTATTA

M = A or C
R = A or G
W = A or T
S = C or T
Y = C or T
H = A or C or T
N = A or G or C or T.

FIG. 4

DNA sequence for human preproparathyroid hormone in plasmid pSSHPTH-10 with flanking sequences.

10 30 50
TATGATGATAACCTGCAAAAGACATGGCTAAAGTTATGATTGTCACTGGCAATTGTT

70 90 110
TCTTACAAAATCGGATGGGAAATCTGTTAAGAAGAGATCTGTGAGTGAAATACAGCTTAT

130 150 170
GCATAACCTGGGAAACATCTGAACTCGATGGAGAGTAGAAATGGCTGCGTAAGAACCT

190 210 230
GCAGGGATGTGCACAATTGTTGCCCTGGAGCTCCTCTAGCTCCAGAGATGCTGGTC

250 270 290
CCAGAGGCCCCGAAAAAAAGGAAGACAAATGTCTTGGTTGAGAGGCCATGAAAAAAAGTCTTGG

310 330 350
AGAGGGCAGACAAAGCTGATGTGAATGTATTAACTAAAGCTAAATCCCAGTGAATAATGAAA

370 390 410
ACAGATATTGTCAAGAGTTCTGCTCTAGACAGTGTAGGGCAACAAATACTGCTGCTAATTG

FIG. 5

DNA sequence coding for
preproparathyroid hormone in pSSHPTH-10 with flanking
sequences, showing the corresponding amino acid
sequence of preproparathyroid hormone.

10 30 50
TATGATGATAACCTGC~~AAAAGACATGGCTAAAGTTATGATTGTCATGTTGGCAATTGTTT~~
Met Ile Pro Ala Lys Asp Met Ala Lys Val Met Ile Val Met Leu Ala Ile Cys Ph

70 90 110
TCTTAC~~AAAATCGGATGGGAAATCTGTTAAGAAGAGATCTGTGAGTGAAATACAGCTTAT~~
e Leu Thr Lys Ser Asp Gly Lys Ser Val Lys Lys Arg Ser Val Ser Glu Ile Gln Leu Me

130 150 170
GCATAACCTGGGAAAACATCTGA~~ACTCGATGGAGAGTAGAATGGCTGCGTAAGAACGCT~~
t His Asn Leu Gly Lys His Leu Asn Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Le

190 210 230
GCAGGATGTGCACA~~ATTGTTGCCCTTGGAGCTCCTCTAGCTCCCAGAGATGCTGGTTC~~
u Gln Asp Val His Asn Phe Val Ala Leu Gly Ala Pro Leu Ala Pro Arg Asp Ala Gly Se

250 270 290
CCAGAGGCCCGAAAAAAGGAAGACA~~ATGTCTTGGTTGAGAGCCATGAAAAAGTCTTGG~~
r Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Glu Lys Ser Leu Gl

310 330 350
AGAGGCAGACAAAGCTGATGTGAATGTATTA~~ACTAAAGCTAAATCCCAGTGAATGAA~~
y Glu Ala Asp Lys Ala Asp Val Asn Val Leu Thr Lys Ala Lys Ser Gln End

370 390 410
ACAGATATTGTCAGAGTTCTGCTCTAGAC~~ACTGTAGGGCAACAATACATGCTGCTAATT~~
AAAGCTCTATT.

Figure 6. Nucleotide sequence of the MF 1-HPTH fusion gene from pS LX5-HPTH1. Nucleotide nos. 1-173 makeup the MF 1 promoter region and 5' noncoding sequence. 174-440 is the MF 1 N-terminal coding sequence. 441-695 is the HPTH sequence obtained from pSSHPTH-10. 696-726 is an HPTH 3' noncoding sequence from pSSHPTH-10. 727-732 is from pUC19. 733-874 is MF 1 3' noncoding sequence and transcriptional termination signal.

730 750 770
TC~~T~~ T~~A~~GAGTCGACTTGT~~T~~CCC~~A~~CTG C~~T~~TTAGCTCGTACAAAATACAATATA~~C~~
90 810 830
TTTCAT~~T~~TCTCCGTAAACAAC~~T~~GT~~T~~CCATGTAATATC~~C~~TTTCTATTTTCGTTT
10 850 870
CGTTACCAACTTACACATACTTATATAGCTAT

0 10 20 30 40 50 60 70 80 90 100

Figure 7. Partial DNA sequence for the plasmid for insertion into yeast in which: [REDACTED] cleotide nos. 1-173 makes the MF 1 promoter region and 5' noncoding sequence. 174-440 is the MF 1 N-terminal coding sequence. 441-695 is an HPTH sequence. 696-726 is an HPTH 3' noncoding sequence from pSSHPTH-10. 727-732 is from pUC19. 733-874 is MF 1 3' noncoding sequence and transcriptional termination signal.

10 10 30
50

AGTGCAAGAAAACCAAAAGCAACAAACAGGTTTGGATAAGTACATATATAAGAGGGCCT

70 90 110
TTTGTCCCATTCAAAATGTTACTGTTCTTACGATTCAATTACGATTCAAGAATAGTTCA

15 130 150 170
AACAAAGAAGATTACAAACTATCAATTTCATACACAATATAAACGACCAAAAGAACATGAGAT

190 210 230
TTCCCTCAATTTCATTGCAGTTTATTGCAGCATCCTCCGCATTAGCTGCTCCAGTCA

250 270 290
ACACTACAAACAGAAGATGAAACGGCACAAATTCCGGCTGAAGCTGTATCGGTTACTCAG

20 310 330 350
ATTAGAAGGGGATTCGATGTTGCTGTTGCCATTCCAACAGCACAAATAACGGGT

370 390 410
TATTGTTATAAATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGTATCTTGG

25 430 450 470
ATAAAAGAGAGGCTGAAGCTWSNGTNWSNGARATHCARYTNATGCAYAAYYTNGGNAARC

490 510 530
AYYTNAAYWSNATGGARMGNGTNGARTGGYTNMGNAARAARYTNCARAYGTNCAYAAYT

550 570 590
TYGTNGCNYTNGGNGCNCCNYTNGCNCCNMNGNGAYGCNGGNWSNCARMGNCCNMGNAARA

610 630 650
ARGARGAYAAYGTNYTNGTNGARWSNCAYGARAARWSNYTNGGNGARGCNGAYAARGCNG

5 670 690 710
AYGTNAAYGTNYTNACNAARGCNAARWSNCARTRRAATGAAAACAGATATTGTCAGAGT

730 750 770
TCTC C GAGTCGACTTTGTTCCCACTG1. TAGCTCGTACAAAATACAATATAC
90 810 830
TTTCACTTCTCCGTAAACAAACCTGTTTCCCATGTAATATCCTTTCTATTTTCTGTTT
10 850 870
CGTTACCAACTTACACATACTTATATAGCTAT, wherein

M = A or C
R = A or G
W = A or T
S = C or G
Y = C or T
H = A or C or T
N = A or G or C or T
15

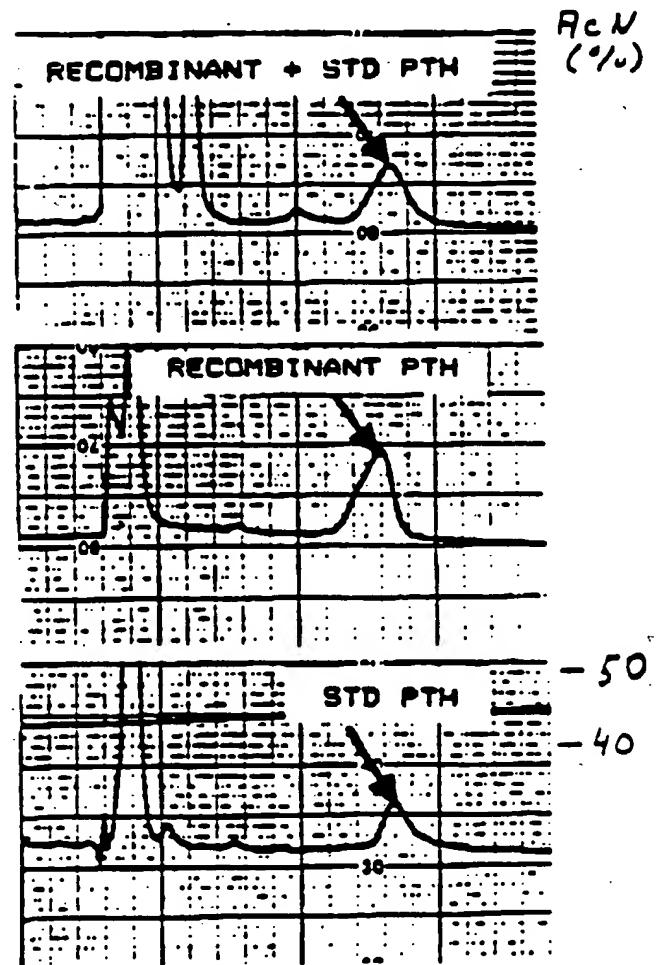
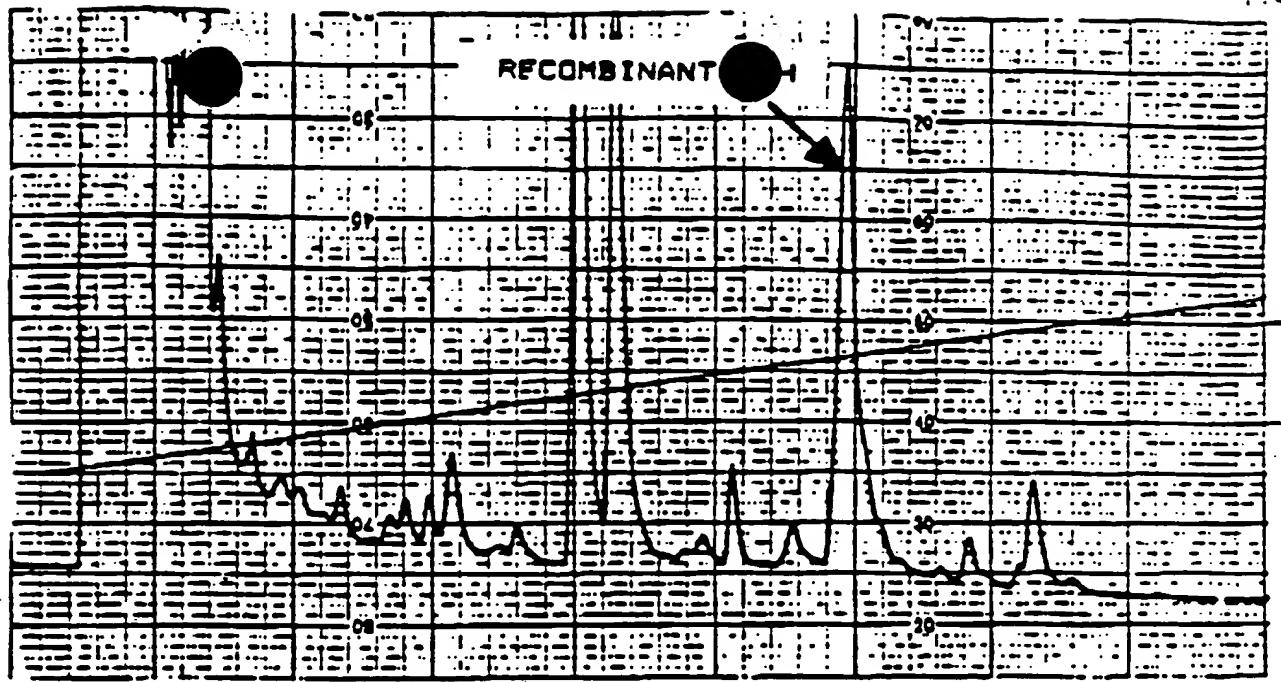


FIG. 9

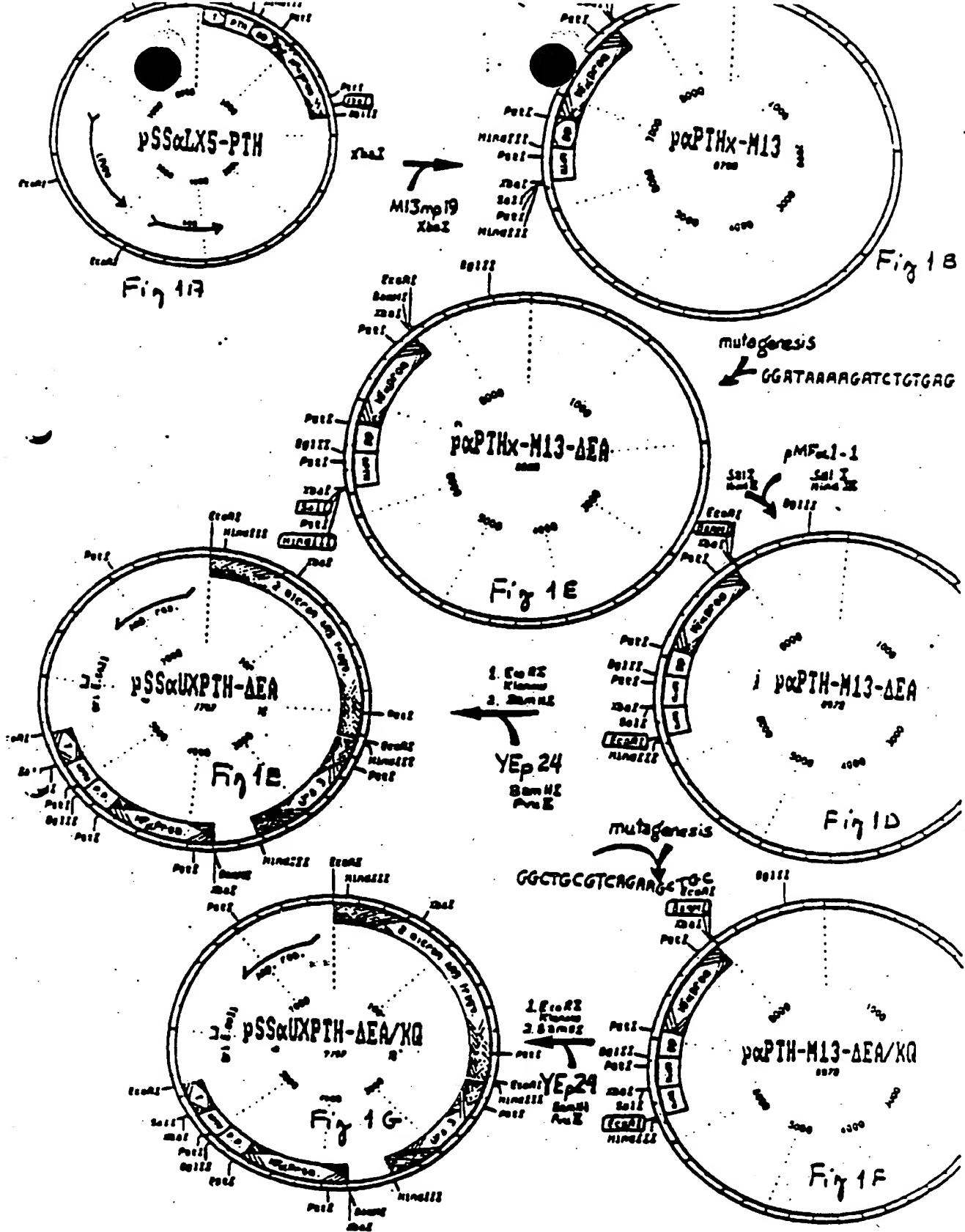


FIG. 10

vitro mutagenesis hPTH

MFα prepro

hPTH

RKK

RKK



hPTH⁰²⁶

RDK

RKK

26

FIG. 11

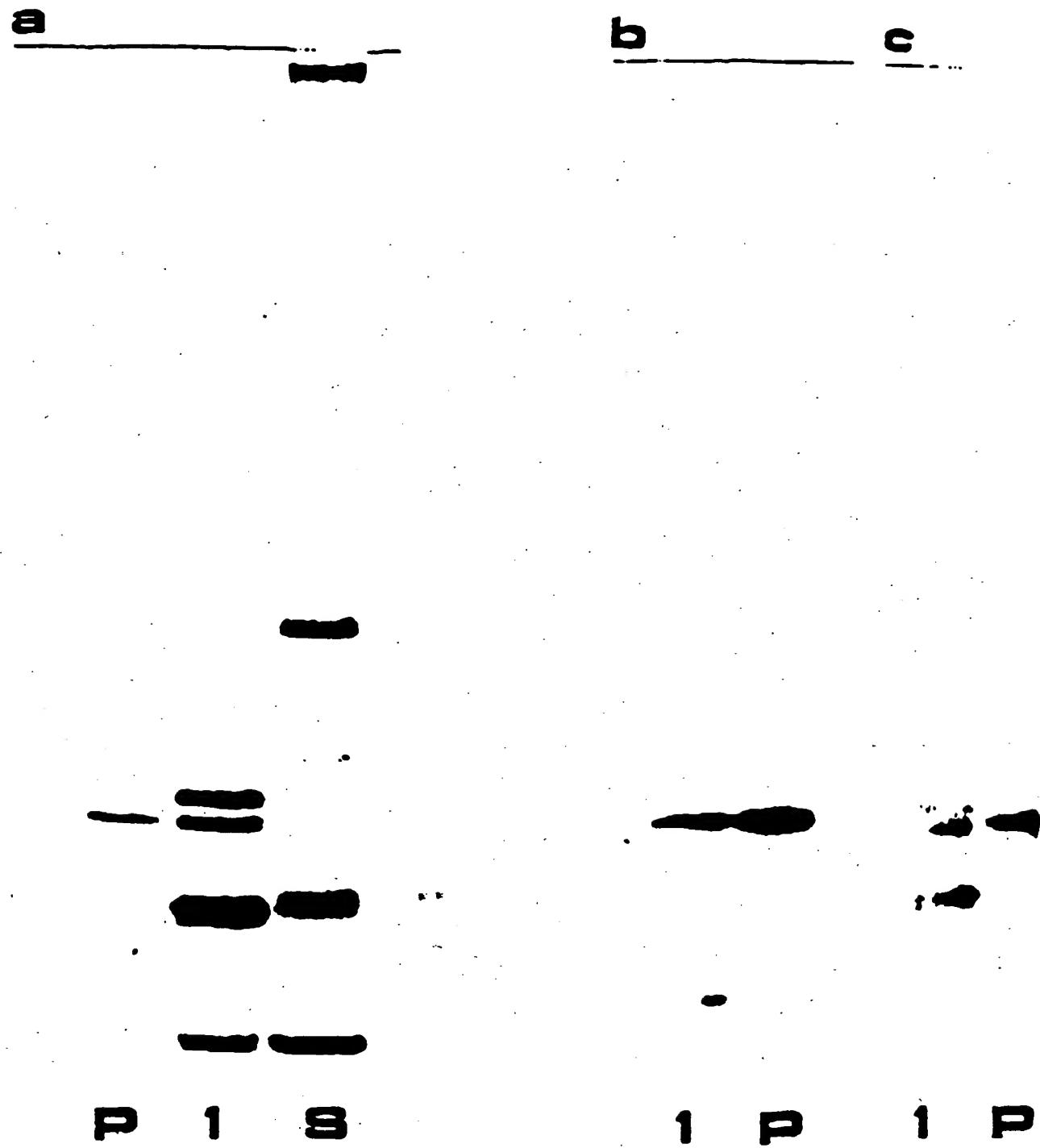


FIG. 8

0 9 8 7 6 5 4 3 2 1

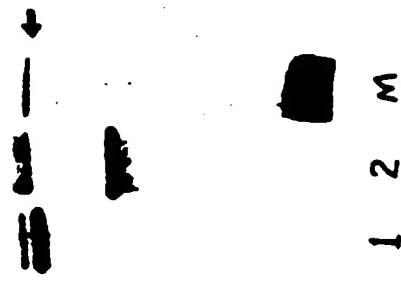


FIG. 12

Elution time

Dose 0.154 mg/mmol

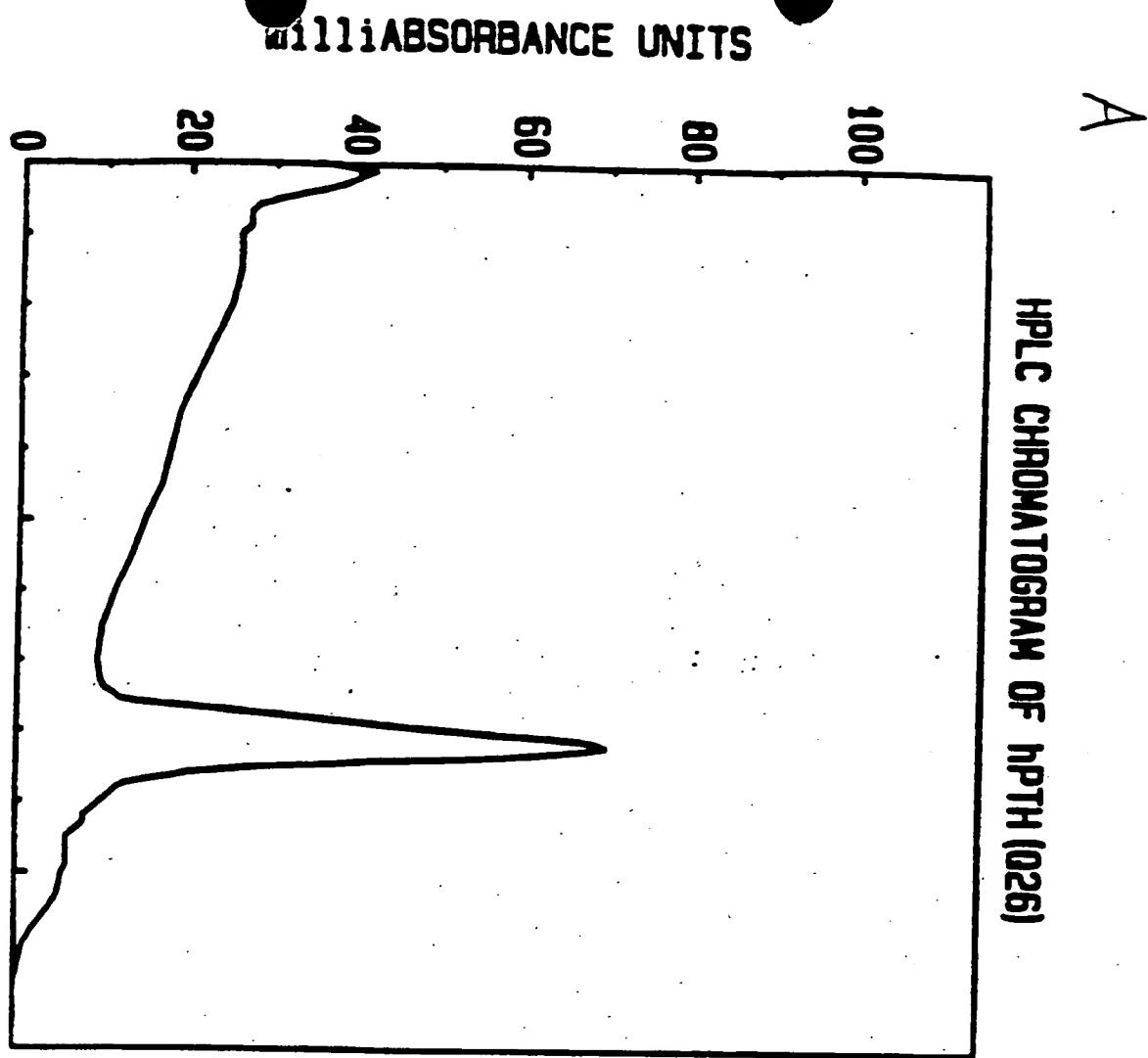


FIG. 13

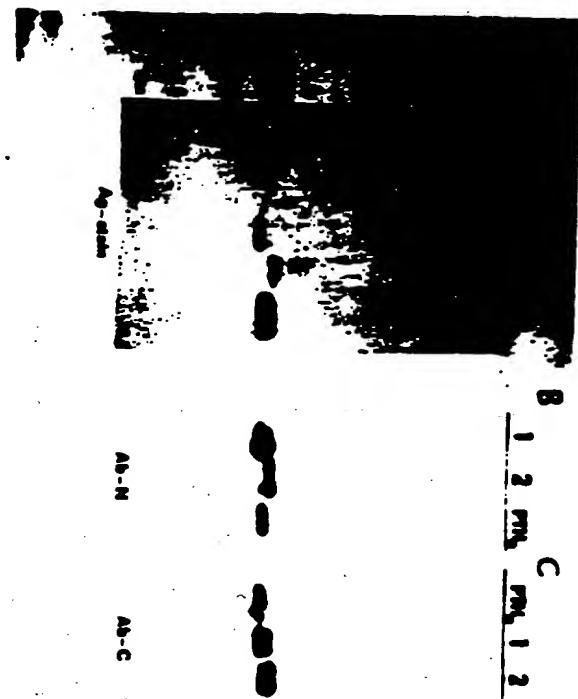


FIG. 14

00562154 - 002000

FIG. 15

